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Antibiotic Resistance and Serotypes of 100 *Streptococcus pneumoniae* Strains Isolated in a Children's Hospital in Barcelona, Spain

CRISTINA LATORRE,* TERESA JUNCOSA, AND ISABEL SANFELIU

Service of Microbiology, Hospital San Juan de Dios, Barcelona, Spain

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A total of 100 *Streptococcus pneumoniae* strains with various penicillin G susceptibilities, isolated in Barcelona, Spain, from different pediatric sources during 1983 and the first 4 months of 1984, were tested for susceptibility to tetracycline, chloramphenicol, erythromycin, clindamycin, vancomycin, and rifampin. The isolates were distributed in nine patterns of antibiotic resistance, and 15 different serotypes were encountered. The high incidence of resistance to multiple antibiotics clearly indicates the need to perform antibiotic susceptibility testing of all pneumococcal isolates with proved pathologic significance to avoid therapeutic failure.

A progressive increase in the number of penicillin-resistant strains of *Streptococcus pneumoniae* (7–10, 12, 13, 19, 24) and in the resistance of these strains to many other antibiotics (7, 12, 16, 18–20, 23, 25), mainly chloramphenicol (2, 5), reported worldwide during the last several years has also been observed in Spain (4) and, in particular, in our geographic area (16, 17).

There have been few studies among the pediatric population in our area. One of them reported a 35.9% incidence of pneumococci of decreased penicillin susceptibility in pharyngeal swabs of healthy carrier children (20). In another study, done in our center (with hospitalized children), 51% of the pneumococcal isolates from different sources had decreased penicillin susceptibility (15).

Of the 100 strains studied here, forty-nine were susceptible to penicillin G (MIC, <0.1 μg/ml), 31 were partially resistant (MIC, 0.1 to 1 μg/ml), and 20 were resistant (MIC, >1 μg/ml). The strains were recovered from different sources (39 from the respiratory tract, 42 from the middle ear, 6 from cerebrospinal fluid, 1 from blood, and 12 from miscellaneous products), all of them from different patients.

The strains studied represented only a part of the pneumococci isolated in our laboratory during the same period, and they were obtained without any selective criteria: the isolates used for this study were those that survived the freezing to which they were subjected from the moment of their isolation until the study was done (305 strains were frozen, and only 100 were recovered).

All frozen strains had previously been screened for penicillin G susceptibility by an agar-disk diffusion test with 1-μg oxacillin disks (1). The resistance rate among strains not recovered after storage was similar to that of strains recovered for the study.

Susceptibility to the following antibiotics was studied: tetracycline, chloramphenicol, erythromycin, clindamycin, vancomycin, and rifampin. Susceptibility tests were performed from a bacterial inoculum whose turbidity was equivalent to a McFarland standard of 0.5. From this suspension, disk diffusion tests were performed on blood agar plates by the modified Kirby-Bauer technique used by Jacobs et al. (11), and microbroth or agar dilution tests were also performed.

TABLE 1. Susceptibility of 100 *S. pneumoniae* strains to the antimicrobial agents tested[a]

Antimicrobial agent	No. of strains inhibited by MIC (μg/ml) of:										
	≤0.06	≤0.12	0.12	0.25	0.50	1	2	4	8	16	≥32
Penicillin G	49		6	1	10	13	16	5			
Tetracycline		15		1	1	1	2	2	13	29	36
Chloramphenicol							22	8	8	34	28
Erythromycin		92		1		1		2			4
Clindamycin		88		3		3		1			5

[a] Strains were susceptible to vancomycin and rifampin at MICs of ≤0.625 and ≤0.015, respectively.

Tetracycline, chloramphenicol, erythromycin, and clindamycin were studied by microbroth dilution with Sensititre plates (Seward Laboratories) (22). A final concentration of 10^5 CFU/ml was obtained by diluting the inoculum in tryptic soy broth. A 50-μl portion of this dilution, supplemented with 5% defibrinated horse blood, was inoculated into each well of the plate. Once inoculated, the plate was incubated for 18 h at 35°C in an aerobic atmosphere.

Vancomycin and rifampin were studied by agar dilution (25). Inocula containing approximately 10^4 organisms were delivered by a Steers replicator onto Mueller-Hinton agar plates supplemented with 5% defibrinated horse blood and

* Corresponding author.

TABLE 2. Resistance and susceptibility patterns and their serotype distribution

Resistance and susceptibility pattern[a]	No. of strains of the following serotype:														
	1	3	5	6	7	9	11	12	14	15	17	19	20	23	24
Resistance															
P							1					3		2	
T			3	2				1	2			4			1
PT												1		1	
TC		1	1	5	1				1		1	4		2	
TEClin												1			
PTC				11		1	1	1	1	1		4		21	
TCEClin				1								1		1	
PTCEClin				1										1	
Susceptibility (PTCEClinVR)	1		4	2		1		2	1	1		2	1	1	

[a] P, Penicillin G; T, tetracycline; C, chloramphenicol; E, erythromycin; Clin, clindamycin; V, vancomycin; R, rifampin.

containing different antibiotic concentrations. Plates were incubated for 18 h at 35°C in an aerobic atmosphere.

Staphylococcus aureus ATCC 25923 was included as a control in all tests.

There was a good correlation between inhibition diameters in the disk diffusion method and MICs obtained by dilution methods.

A total of 78 strains showed diminished susceptibility to tetracycline (65 of them with MICs of >12 μg/ml), 62 were less susceptible to chloramphenicol (28 of them with MICs of >25 μg/ml), and 6 were less susceptible to erythromycin and clindamycin (4 of them with MICs of >8 μg/ml for erythromycin and all 6 with MICs of >2 μg/ml for clindamycin). No strain was resistant to rifampin or vancomycin, with MICs of ≤0.015 μg/ml for the former and ≤0.625 μg/ml for the latter (Table 1).

A total of 16 strains were susceptible to all antibiotics tested, and the remaining 84 strains were distributed in eight resistance patterns, the most common of them being associated resistance to penicillin G, tetracycline, and chloramphenicol (41 strains); 16 strains were resistant to tetracycline and chloramphenicol; 13 were resistant to tetracycline; 6 were resistant to penicillin G; 3 were resistant to tetracycline, chloramphenicol, erythromycin, and clindamycin; 2 were resistant to penicillin G and tetracycline; 2 were resistant to penicillin G, tetracycline, chloramphenicol, erythromycin, and clindamycin; and 1 was resistant to tetracycline, erythromycin, and clindamycin.

All strains were serotyped to observe whether there was any relationship between serotype, pathogenicity, and resistance level. The 100 strains belonged to 15 serotypes or serogroups (1, 3, 5–7, 9, 11, 12, 14, 15, 17, 19, 20, 23, 24), the most frequent being serogroup 23 (3, 6, 16) (29 strains), followed by serogroups 6 (22 strains) and 19 (20 strains).

Table 2 correlates resistance patterns and the number of strains of each serotype or group manifesting the pattern. It is noteworthy that the greatest number of strains of the penicillin G-tetracycline-chloramphenicol resistance pattern belonged to serogroup 23 (21 strains).

The 35 strains from the upper respiratory tract without a proved pathologic role (they were part of the normal flora of the area, together with other normally saprophytic microbes) were distributed among nearly all serotypes. In the same way, the strains with pathologic significance also had a uniform distribution among serotypes or groups. The most frequent serotypes among strains from the upper respiratory tract (6, 19, 23) were also the most frequent among strains with a pathologic role.

There was no relation between the source of the pneumococcal strains and their patterns of resistance to antimicrobial drugs (Table 3). The most frequent pattern among strains isolated from the upper respiratory tract (penicillin G-tetracycline-chloramphenicol) was also the most frequent pattern among strains isolated from sites where they were considered to be the cause of infection.

Of the 100 strains studied by us, 78 were tetracycline resistant, a result that is similar to others previously reported (16), and 62 were chloramphenicol resistant, which is alarmingly higher than data reported until now in our geographic area (5, 16). The prevalence of erythromycin and clindamycin resistance was low, as previously reported by others (10, 16, 18). All strains studied by us were susceptible to vancomycin and rifampin; thus, vancomycin (as a single treatment [10]) and rifampin (in association with other antibiotics [14]) can be considered valuable alternatives in the treatment of severe pneumococcal infections.

Most penicillin-resistant strains were also resistant to tetracycline and chloramphenicol. This fact makes inadvisable the use of these two antibiotics in the treatment of penicillin-resistant pneumococcal infections.

Of the 51 penicillin-resistant strains, 25 belonged to serogroup 23, alarming in view of the high incidence of penicillin resistance of this serogroup in our geographic area.

TABLE 3. Sources of strains and their resistance and susceptibility patterns

Resistance and susceptibility pattern[a]	No. of strains from the following source[b]:					
	URT	LRT	ME	CSF	Blood	M
Resistance						
P	3		1	1		1
T	4		8	1		
PT			1		1	
TC	7		8			1
TEClin			1			
PTC	16	4	12	3		6
TCEClin	2		1			
PTCEClin			2			
Susceptibility (PTCEClinVR)	3		8	1		4

[a] See footnote to Table 2 for definition of abbreviations.

[b] URT, Upper respiratory tract; LRT, lower respiratory tract; ME, middle ear; CSF, cerebrospinal fluid; M, miscellaneous products.

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